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(FILE 'HOME' ENTERED AT 19:38:35 ON 08 JUN 2005)

FILE 'BIOSIS, MEDLINE, CAPLUS' ENTERED AT 19:38:48 ON 08 JUN 2005

L1	7184 S GPCR
L2	388 S L1 AND LIBRARY
L3	25 S L2 AND MUTATION
L4	8 S L3 AND SCREEN
L5	4 DUP REM L4 (4 DUPLICATES REMOVED)

L5 ANSWER 1 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
 DUPLICATE 1

TI Random mutagenesis of the M3 muscarinic acetylcholine receptor expressed
 in yeast - Identification of second-site **mutations** that restore
 function to a coupling-deficient mutant M3 receptor.

PY 2005

SO Journal of Biological Chemistry, (February 18 2005) Vol. 280, No. 7, pp.
 5664-5675. print.
 CODEN: JBCHA3. ISSN: 0021-9258.

TI Random mutagenesis of the M3 muscarinic acetylcholine receptor expressed
 in yeast - Identification of second-site **mutations** that restore
 function to a coupling-deficient mutant M3 receptor.

AB The M, muscarinic receptor is a prototypical member of the class A family
 of G protein-coupled receptors (**GPCRs**). To gain insight into
 the structural mechanisms governing agonist-mediated M, receptor
 activation, we recently developed a genetically modified yeast strain
 (*Saccharomyces cerevisiae*) which allows the efficient screening of large
libraries of mutant M3 receptors to identify mutant receptors with
 altered/novel functional properties. Class A **GPCRs** contain a
 highly conserved Asp residue located in transmembrane domain II (TM II;
 corresponding to Asp-113 in the rat M3 muscarinic receptor) which is of
 fundamental importance for receptor activation. As observed previously
 with other **GPCRs** analyzed in mammalian expression systems, the
 D113N point **mutation** abolished agonist-induced receptor/protein
 coupling in yeast. We then subjected the D113N mutant M, receptor to
 PCR-based random mutagenesis followed by a yeast genetic **screen**
 to recover point **mutations** that can restore G protein coupling
 to the D113N mutant receptor. A large scale screening effort led to the
 identification of three such second-site suppressor **mutations**,
 R165W, R165M, and Y250D. When expressed in the wild-type receptor
 background, these three point **mutations** did not lead to an
 increase in basal activity and reduced the efficiency of receptor/G
 protein coupling. Similar results were. . . are located at the
 cytoplasmic ends of TM III and TM V, respectively, are also highly
 conserved among class A **GPCRs**. Our data suggest a
 conformational link between the highly conserved Asp-113, Arg-165, and
 Tyr-250 residues which is critical for receptor. . .

IT Major Concepts

IT Biochemistry and Molecular Biophysics; Methods and Techniques

IT Chemicals & Biochemicals

IT G-protein-coupled receptors [**GPCRs**]: Class A family,
 transmembrane domain II, transmembrane domain III, transmembrane domain
 V; M-3 muscarinic receptor

IT . . . & Equipment

IT PCR [polymerase chain reaction]: genetic techniques, laboratory
 techniques; random mutagenesis: genetic techniques, laboratory
 techniques

IT Miscellaneous Descriptors

IT point **mutation**

L5 ANSWER 2 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

TI A high throughput cell-based **screen** for identification of
 putative Alzheimer's disease modifying drugable genes that modulate
 amyloid levels.

PY 2003

SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003)
 Vol. 2003, pp. Abstract No. 445.11. <http://sfn.scholarone.com>. e-file.
 Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New
 Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.

TI A high throughput cell-based **screen** for identification of
 putative Alzheimer's disease modifying drugable genes that modulate
 amyloid levels.

AB Genetic linkage studies revealed segregation of **mutations** in APP
 and in APP-processing genes PS1 and PS2 with Alzheimers disease pathology
 and clinical phenotype. These findings underscored the. . . Our state
 of the art arrayed adenoviral platform allows automated, highly efficient
 induction of single genes into mammalian cells. Pre-selected

libraries of adenoviruses holding cDNAs or siRNA sequences of drugable genes are applied that knock in or knock down genes, respectively.. . . secreted Abeta levels reproducibly, both in the knock-in and knock-down approach. Genes of different drugable classes are screened, such as **GPCRs**, NHR, kinases and others. Up to now, 3 new **GPCRs** are identified that upon overexpression modulate Abeta levels in conditioned medium in a cell specific manner. In conclusion, combining these. . .

IT . . .
and mental disorders, nervous system disease
Alzheimer Disease (MeSH)

IT Diseases
infection: infectious disease
Infection (MeSH)

IT Chemicals & Biochemicals
A-beta1-42; BACE; **GPCR**; NHR; PS1; PS2; amyloid; genes; siRNA

L5 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
TI MECHANISMS OF DELTA OPIOID RECEPTOR ACTIVATION.
PY 2002

SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002)
Vol. 2002, pp. Abstract No. 515.7. <http://sfn.scholarone.com>. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience.
Orlando, Florida, USA. November 02-07, 2002. Society for Neuroscience.
AB. . . (CAM) receptors. We have optimized PCR conditions to randomly
mutate the entire hDOR cDNA under conditions that statistically introduce
one point-**mutation** per receptor molecule. We have transiently
expressed the receptor **library** into HEK 293 cells and used a
high-throughput reporter gene assay in conjunction with the inverse
agonist IC1174864 to identify. . . receptors. Out of a screening of
3000 clones, we obtained several mutant receptors and identified the
nature and localization of **mutations** by DNA sequencing. Mutants
were also transfected into COS cells to confirm constitutive activity
using another functional assay (GTPgammaS). Interestingly,
mutations are organized in discreet microdomains and allow to
speculate on possible mechanisms for hDOR activation using 3D-modelling.
This strategy offers. . . draw a general picture of receptor
activation. Both the approach and some of the conclusions may be
applicable to other **GPCRs**. Mutant receptors will be useful to
screen for compounds with inverse agonist properties.

IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics); Neurology
(Human Medicine, Medical Sciences)

IT Chemicals & Biochemicals
DNA; **GPCR**; IC1174864; constitutively active mutant receptor:
expression; delta opioid receptor: activation; h-delta opioid receptor
cDNA: activation

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DUPLICATE 2

TI A limited spectrum of **mutations** causes constitutive activation
of the yeast alpha-factor receptor.
PY 2000

SO Biochemistry, (June 13, 2000) Vol. 39, No. 23, pp. 6898-6909. print.
CODEN: BICHAW. ISSN: 0006-2960.

TI A limited spectrum of **mutations** causes constitutive activation
of the yeast alpha-factor receptor.

AB Activation of G protein coupled receptors (**GPCRs**) by binding of
ligand is the initial event in diverse cellular signaling pathways. To
examine the frequency and diversity of **mutations** that cause
constitutive activation of one particular **GPCR**, the yeast
alpha-factor receptor, we screened **libraries** of random
mutations for constitutive alleles. In initial **screens**
for mutant receptor alleles that exhibit signaling in the absence of added
ligand, 14 different point **mutations** were isolated. All of
these 14 mutants could be further activated by alpha-factor. Ten of the
mutants also acquired the. . . of endogenous alpha-factor present in
MATa cells. The strongest constitutively active receptor alleles were

recovered multiple times from the mutational libraries, and extensive mutagenesis of certain regions of the alpha-factor receptor did not lead to recovery of any additional constitutive alleles. Thus, only a limited number of mutations is capable of causing constitutive activation of this receptor. Constitutive and hypersensitive signaling by the mutant receptors is partially suppressed. . .